A simulated avalanche search and rescue mission induces temporary physiological and behavioural changes in military dogs.

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Running title: Physiological and behavioural changes in avalanche rescue dogs

Abstract

Saving human lives is of paramount importance in avalanche rescue missions. Avalanche military dogs represent an invaluable resource in these operations. However, their performance can be influenced by several environmental, social and transport challenges. If too severe, these are likely to activate a range of responses to stress, which might put at risk the dogs' welfare. The aim of this study was to assess the physiological and behavioural responses of a group of military dogs to a Simulated Avalanche Search and Rescue mission (SASR). Seventeen avalanche dogs from the Italian Military Force Guardia di Finanza (SAGF dogs) were monitored during a simulated search for a buried operator in an artificial avalanche area (SASR). Heart rate (HR), body temperature (RBT) and blood samples were collected at rest the day before the trial (T0), immediately after helicopter transport at the onset of the SASR (T1), after the discovery of the buried operator (T2) and 2 hours later (T3). Heart rate (HR), rectal body temperature (RBT), cortisol, aspartate aminotransferase (AST), creatine kinase (CK), non-esterified fatty acids (NEFA) and lactate dehydrogenase (LDH) were measured. During the search mission the behaviour of each SAGF dog was measured by focal animal sampling and qualitatively assessed by its handler and two observers. Inter-rater agreement was evaluated. Snow and environmental variables were also measured.

All dogs successfully completed their search for the buried, simulated victim within 10 minutes. The SASR was shown to exert significant increases on RBT, NEFA and cortisol ($P<0.001$), CK and HR ($P<0.01$), AST and LDH ($P<0.05$). These indicate the activation of a response to stress probably induced by the addition of factors such as helicopter transport, disembarking, and the search and rescue exercise. However, changes were moderate and limited over time, progressively decreasing with complete recovery at T3 except for sera cortisol that showed a slightly slower decline. More time walking within the search was related to lower RBT, conversely to walking. Standing still with head up and exploring with head-up were inversely related with HR. Agreement between handler and observers' opinions on a dog's search mission ability was found only for motivation, signalling behaviour, signs of stress and possessive reward playing. More time signalling was related to shorter search time. In conclusion, despite extreme environmental and training conditions only temporary physiological and behavioural changes were recorded in the avalanche dogs. Their excellent performance in successful simulated SASR may be attributable to extensive training and good dog-handler relationships. Simulated SASR did not seem to impair SAGF dogs' performance or welfare.

Keywords:

AVALANCHE MISSION; DOG PHYSIOLOGY; DOG BEHAVIOUR; CORTISOL; HEART RATE; SEARCH AND RESCUE DOGS

Glossary

GdF: Italian Military Force Guardia di Finanza

SAA: Simulated Avalanche Area

SAGF dogs: Avalanche Search and Rescue Military GdF dogs

SASR: Simulated Avalanche Search and Rescue mission

1. Introduction

Dogs represent an irreplaceable resource in the case of avalanche disasters because of their ability to pinpoint rapidly the location of victims buried beneath the snow [1]. Because of their keen olfactory ability, dogs have been employed not only for locating disaster survivors, lost persons or terrorists [2], but also cadavers and their resting places [3], drugs and explosives [2][4] and wildlife [5]. The success of avalanche rescue missions depends upon a wide range of factors, which can influence the dogs' ability to detect a human scent (i.e., air temperature, direction of the wind, snow composition and burial depth). In addition, factors such as training context [6], physical activity [7], environmental challenges [8] and quality of the relationship between handler and dog have been shown to influence dogs' efficiency and welfare [9]. The level of fitness is another potential factor of importance in avalanche rescue dogs, because during a search they often need to cover large search areas in extremely harsh climatic conditions. In the case of avalanche, the speed of rescue operations is of utmost importance: a victim's chance of survival dramatically declines with time following snow burial [10][11][12]. For this reason, rescue dog-handler units are mostly transported by helicopter to avalanche rescue zones. Harsh climatic conditions, transport challenges, and highly demanding physical activity involved in avalanche missions are likely to be stressful for search and rescue dogs. These circumstances may be cause of delay in the discovery of disperse victims and, over the time, might compromise dogs' welfare. Notwithstanding, so far few studies have been conducted on search and rescue dogs [13][14][15].

Previous studies have measured physiological responses induced by a range of potentially stressful situations in dogs, such as living in a shelter [16][17], agility work [18], training and outdoor conditions [13][19]. Cortisol has been indicated as a major indicator of altered physiological states that strongly correlates with stress [16][17]. It has been used to detect poor dog welfare [16][20], especially when the stressor was an immediate challenge, because it is known to facilitate energy release [21]. High levels of this hormone may indicate marked stress during cold and/or heat stress, after physical exercise and performance in a competition

[13][19]. Variability of some physiological variables (i.e. heart rate and body temperature) has also been considered as useful indicators for assessing the response to stress in the dog [22][17]. Heart rate (HR) monitoring has been widely used as an accessible, quantifiable, physiological measure underlying emotional responses in dogs [23][24]. Increased body temperature, not linked to a state of disease, have also been used to as a measure of emotional response to stress in other animals [25]. In addition, increases in blood concentrations of creatine kinase (CK), aspartate aminotransferase (AST) and non-esterified fatty acids (NEFA), as well as lactate dehydrogenase (LDH) have been used to evaluate muscle effort at the end of a physical stress [26][14]. Behaviour can also differ in dogs when they are challenged in different situations [27], being an expression of different coping strategies [28]. In order to assess behavioural changes both quantitative and qualitative methods can be used. Quantitative methods of assessing behaviour are a way to measure objectively specific aspects of the dogs' behaviour [29] and have been previously used to measure behavioural responses in working dogs [8][20][30]. Modifications of the proportion of time spent in performing specific behaviours during the search activity may indicate possible predictive behaviours associated with enhanced mission success. However, to improve the success rate of search and rescue missions, it is of utmost importance that dogs' handlers are able to judge empirically the ultimate search performance of their dogs, including potential critical aspects, which might cause a delay in the discovery of the missing victim. A qualitative method for assessing search dog search and ability has been previously successfully exploited [31]. It relayed upon agreement between trainers rating their dogs' behaviour while searching, and results were comparable with those of a scientist expert on dog behaviour.

Notwithstanding a growing body of research examining stress in the dog, very few studies have been focused on the impact of search and rescue training and work [14][32][33]. The aim of this study was to analyse the physiological and behavioural responses of avalanche military dogs to a simulated search and rescue mission, to investigate whether it might elicited stress or influenced their performance. To the best of our knowledge, this is the first attempt to measure the effect of a search and rescue mission on avalanche military dogs. We adopted a multidisciplinary approach including the measurement of physiological and behavioural changes in the dogs together with a qualitative assessment of the dogs' performance by means of the trainers' subjective ratings at the end of the search missions. This group of avalanche military dogs was homogeneous in terms of physical variables and search performance ability, thus enhancing experimental reproducibility.

2. Materials and Methods

All experimental procedures in the present study were in agreement with the Ethical Committee of Perugia University. This research complies with the laws of the Italian Ministry of Health. There is a standing agreement between the Italian Military Force of Guardia di Finanza (GdF) and the Department of Veterinary Medicine of Perugia for the ethical testing and study of GdF working dogs.

2.1. Experimental conditions

This study is part of a broader collaborative project among the Italian Military Force of GdF, the Department of Veterinary Medicine (Perugia University), the Agenzia Regionale per la Prevenzione e Protezione Ambientale del Veneto (ARPAV) and the Istituto per la Dinamica dei Processi Ambientali del Consiglio Nazionale delle Ricerche (IDPA-CNR-Venice University) aimed at identifying limiting factors affecting the success of avalanche search and rescue missions. The study was carried out during a 2nd retraining course (February 2012) for the Avalanche Search and Rescue Military Dog-Handler Units (SAGF Units) at the GdF Alpine School of Passo Rolle (Trento, Italy). During the course the SAGF Units were trained using two sessions of a Simulated Avalanche Search and Rescue mission (SASR) carried out on different days, with half the group tested on one day, the other half on the next. The avalanche victims were played by military personnel, safely buried beneath the snow prior to the arrival of the SAGF, with the burial site then masked to match the remaining search area.

A search area of 1 hectare was established at an altitude of 2170 m and prepared to resemble an avalanche fall environment (Simulated Avalanche Area: SAA). The snow was moved and compressed by a snow-cat. Three identical pits (1.5m deep, 50 x 100cm wide) were dug, carefully avoiding contaminating these areas with human scent. In each of the trials the 'victim' was entirely buried in snow to a depth of 1m in one of the three pits, chosen at random, the snow further compressed by the snow-cat following initial burial. For safety reasons a hole for air turnover was left in the pits, but not open to the air, and 'victims' were limited to a maximum of 60 minutes burial.

During each SASR, a team of experts from the ARPAV continuously monitored meteorological conditions. They collected data through a small Oregon Scientific portable station (Oregon Scientific Wireless Weather Station, Tualatin, OR, USA) installed at a campsite nearby the SAA. During the experimental period, the environmental air temperatures ranged from -8.5 to -10.4°C with 28% humidity and a relative wind-chill of -29°C.

2.2. Subjects

The study used 17 Avalanche Search and Rescue Military GdF Dogs (SAGF dogs); fifteen male and two female, aged from 1 to 8 years, and belonging to various breeds (eight German shepherd, six Belgian Shepherd Malinois, three Border Collies) (Table 1). All SAGF dogs were physically (i.e. X-ray negative for hip dysplasia and found in good health from a veterinarian) and behaviourally tested (i.e. absence of behavioural pathologies identified by a veterinary behaviour consultant) to certify their suitability for training work. The SAGF dogs came from different areas of Italy and lived with their handlers all year round. All of them arrived at Passo Rolle a week before the beginning of the study, to allow time for adaptation to the new environment. During both the adaptation and the experimental period the SAGF dogs were individually kennelled in indoor pens (2.9 x 2.1 x 2.3m) at a station beside the GdF Alpine School at Passo Rolle and fed with a commercial, dry dog food. All SAGF dogs had been operational in a search and rescue working dog-handler unit for a minimum of 1 year. All dogs had been previously trained to be transport by helicopter. Their handlers had at least 2 years of working experience with search and rescue dogs at the start of the experiment.

2.3. ***Experimental design***

Each SAGF dog was individually tasked with a full SASR. Baseline samples (T0) were collected from the SAGF dogs outside their living pen, the day before SASR, between 08.00 and 09.00 h. The SASR protocol consisted of: in turn, and one at a time starting from 09.00 h, each SAGF Unit, equipped with pulley harness, was loaded into a helicopter and transported for approximately 8 minutes to the area above the SAA. Here, the SAGF dog's handler lowered himself and the dog, safely fastened to his harness, from the hovering helicopter to the ground. Upon arrival, the SAGF Unit moved to the study sampling station, where the GdF veterinarian subjected the dog to the first HR, rectal body temperature (RBT) and blood sample collection (T1) (Fig. 1). Then, the SAGF Unit began the SASR. During the SASR the handler could vocally encourage the dog, moving with it on the SAA at a variable distance. When the dog signalled the location of the 'victim', the handler rewarded the dog by playing with it for a maximum of 3 min. Then, the SAGF Unit returned to the sampling station and the dog was submitted to the second HR, RBT and blood collection (T2). A maximum of 10 min of SASR time was allowed for each SAGF Unit to locate the 'victim'. Considering the 3-min reward time and the 1–2 min required to move to the sampling point, the T1 to T2 sampling interval ranged from 6 up to 15 min. Following T2 sampling, the SAGF handler walked and skied alongside his dog to return him back to his living pen and to let him rest. There, just outside the SAGF dog pen, following 2 hours from T2, the third HR, RBT and blood collection was carried out (T3) (Fig. 1).

2.4. Physiological data

The procedure to collect HR, RBT and blood at each sampling time (T0, T1, T2 and T3) from the SAGF dogs is described in more detail: Each handler asked his SAGF dog to stand and stay still for 1 min, allowing the GdF veterinarian to measure HR and RBT whilst the handler gently manipulated and distracted the dog. HR, in beats per minute, was measured by counting the number of heart beats within a 30 second time window and multiplying it by two. A stethoscope was used (Classic II S.E., 3M™ Littmann®). RBT, in °C, was measured with a digital thermometer (MB TERMO7126500, Reckitt Benckiser SpA, Milano, Italia) gently inserted in the rectum. Following RBT measurement, the handler asked the dog to sit and then gently held it while the veterinarian collected the blood sample (approximately 1mL) from the radial vein using a syringe. Blood collected was rapidly transferred to a glass tube, then centrifuged at 3800 rpm and sera stored at –20°C until analysis. HR, RBT and blood sampling time ranged from 2 to 3 min. During this period, the SAGF dogs did not display any behavioural signs of stress or anxiety but remained calm and attentive.

Sera were analysed for cortisol, CK, AST, LDH and NEFA. Serum cortisol concentrations were assayed in duplicate with the commercially available ^{125}I Cortisol Radioimmunoassay RIA kit (Beckman Coulter®), following the standard procedure. The measurement range was from 10 to 2000 nM. The intra-assay and inter-assay coefficients of variation (CV) were 5.8% and 9.2% respectively. The antibody used in the immunoassay is highly specific for cortisol, with extremely low cross reactivities reported in human samples against other naturally occurring steroids (Aldosterone, Corticosterone, Cortisone, 11-Desoxycortisol, Progesterone, etc.). All samples were processed within a single assay. The concentrations of CK, AST, LDH and NEFA were measured using the automatic bio-analyser AU 400 Olympus®. The analytical methods and reagents (CK, AST and LDH: Beckman Coulter©; NEFA: Randox©) and measure units (CK, AST and LDH: U/L; NEFA: µmol/L) were those designed for this instrument.

2.5. Behavioural observations

An operator located on a nearby slope giving good overall view, filmed the SAGF dogs during the SASR period (from T1 to T2) using a Digital Video Camera Recorder (DCR-SR58, Sony®). For the behavioural analysis, a range of predefined behavioural categories was used [20], modified to the experimental conditions (Table 2). Behaviour variables were mutually exclusive. Behaviour categories were analysed in terms of duration or frequency of occurrence, in relation to the patterns seen in the dog. Behaviours of relatively long duration were considered states and measured as total duration (digging, run, walk, explore head down, explore head up, still head down, still head up - Table 2). Behaviours of relatively short duration were considered events and measured as frequency of occurrence (contact, digging, intensive digging, play, sign of stress - Table 2). We defined as signs of stress a miscellaneous of behaviours (panting, barking, paw-lifting, snout and lip-licking, circling, body shaking, yawning) that have been associated to stress in previous studies [34][35][36][17]. Duration was measured by continuous focal animal sampling, frequency as total number of occurrence of the behaviour within the SASR period. A single operator recorded behavioural data from all the videos after the event. Intra-observer reliability exceeded 90% for all behavioural categories.

2.6. Trainers' behavioural assessment

At the end of the SASR, each SAGF handler, together with an additional two external observers (military experts on training avalanche search and rescue dogs), all independently, qualitatively assessed the search performance of the dog. For this subjective assessment, all three trainers rated the SAGF dogs for 14 different attributes considered to be important in search and rescue military dogs (Table 3). For clarity, we defined: *possessive reward playing,* when the dog was playing with the reward but was reluctant to return it to the handler; *collaborative reward playing,* when the dog was playing to leave and take again the reward with the handler; *sign of stress* when the dog showed at least one of the stress-related behaviours described in Table 2. We differentiate the attributes *marking* and *signalling* according to the behaviour of the dog: we defined as *marking* when the dog was digging for less then 5 seconds and not barking to explore a point, *signalling* when the dog was digging for longer then 5 seconds and was barking to indicate the location of the 'disperse victim'. These 14 attributes were chosen taking into account the general opinion and suggestions given by all SAGF military trainers combined with characteristics highlighted by previous research [37][30]. Similarly to the behavioural assessment approach suggested by Rooney et al., [31], the trainers rated each of nine characteristic of the dog as either: 1, extremely low; 2, low; 3, intermediate; 4, high; or 5, extremely high, whereas for the remaining five characteristics they provided a Yes/No answer (Table 3)..

2.7. *Statistical analysis*

A simple polynomial was fitted to obtain an overall summary across all dogs of the response over time of each of the physiological variables measured. The polynomials were fitted using the statistics package MLwiN [38], which allowed the repeated measurements on each dog to be properly modelled. A polynomial of increasing order was tested in the regression model of each variable and retained when the highest order was of $P \leq 0.05$. Inter-rater agreement was evaluated by means of Cohen's Kappa using the statistics package Stata v12 (StataCorp LP). All behaviour scores were assessed between each possible pair of assessors.
The proportion of time spent carrying out of the eight different behaviours was calculated from the total search time (including the last 10 seconds of digging). The effect of the proportion of the time spent carrying out each of the eight behaviours were tested, using multiple regression, for their effects on each of temperature, total heart rate and total cortisol at time T2. Temperature and heart rate and cortisol at T1 were included in each of the three regressions, respectively, to adjust to T1 as a baseline.

3. Results

Physiological responses

All SAGF dogs successfully completed the SASR within 10 minutes (Fig. 2). SASR significantly affected all measured variables (Fig. 3). The overall line fitted for RBT data showed an increase from T0 (38.51 °C ± 0.09) (mean ± SD) to T1 (39.13 °C ± 0.12) remaining approximately constant at T2 (39.17 °C ± 0.15), then falling back to initial values at T3 (38.59 °C ± 0.15) (P = 0.000).

A trend (P = 0.007) was observed for the overall line fitted for HR data, that showed a steady, linear decline from T0 (116.20 bpm ± 5.03) to T3 (97.65 bpm ± 5.54). However, inspection of the responses for individual dogs showed that for some subjects the HR rose from T0 to reach a peak at T1 (110.02 bpm ± 5.54), then declining to T2 (103.84 bpm ± 3.91).

The overall line fitted for sera cortisol concentrations increased throughout the period from T0 (16.43 ng/mL ± 2.17) to T1 (32.50 ng/mL ± 5.91), and to T2 (38.78 ng/mL ± 6.89), afterwards slightly declining to T3 (35.27 ng/mL ± 5.29) (P = 0.0003).

The overall line fitted for CK data, showed an increase in T1 (181.13 U/L ± 86.89) and T2 (200.71 U/L ± 30.06) compared T0 (122.34 U/L ± 10.10) and decreased a T3 (181.09 U/L ± 18.86) (P = 0.004).

A linear, progressive and incremental response from T0 (31.45 U/L ± 1.04), T1 (37.34 U/L ± 9.78) and T2 (43.22 U/L ± 9.37) to T3 (49.10 U/L ± 7.49) was recorded for AST (P = 0.016).

The overall line fitted for LDH data, similarly to CK, showed the greatest increase in activity from T0 (129.39 U/L ± 15.62) to T1 (196.79 U/L ± 29.62) and T2 (210.02 U/L ± 28.51) and decreasing at T3 (169.06 U/L ± 7.00) (P = 0.024).

The overall line fitted for NEFA data mirrored the response time course of CK and LDH, increasing from T0 (509.22 umol/L ± 97.34) to T1 (994.15 umol/L ± 141.03) and reaching a peak to T2 (1128.90 umol/L ± 201.69), then declining to T3 (913.58 umol/L ± 143.00) (P = 0.001).

Behaviour – Quantitative assessment

The duration (including the last 10 seconds of digging) of the search for the individual SAGF dogs is shown in Fig. 2. All dogs successfully found the buried 'victim' within 600 seconds, many of them (8 out of 17) completing their search within 100 to 200 seconds. Search time ranged from a minimum of 127 seconds to a maximum of 564 seconds. The duration of time spent performing different behaviours, as well as the number of occurrences during the search is reported in Figures 4. On average, SAGF dogs spent most time exploring (37% with the head down and 12% with the head up), 13% of the time running, 11% digging, 5% walking, and staying still (6% with the head down and 3% with the head up). For 13% of the time the behaviour of the SAGF dogs was not visible. No significant relationships were found for duration or frequency of analysed behaviours in relation to search time (to discovery).

There was a significant effect of the proportion of time spent walking and a trend for an effect of the proportion of time spent running on RBT T2 (adjusted for RBT at T1). The higher the proportion of walking, the lower the RBT ($b = -7.622$, $P = 0.020$), and higher proportion of running, the higher the RBT ($b = 2.099$, $P = 0.064$).

There was a significant effect of the proportion of time spent standing still with head up and the proportion of time spent exploring with head-up on overall HR at T1 and T2. The higher the proportion of time spent standing still with head up within the search the lower the HR ($b = - 481.7$, $P = 0.006$). The higher the proportion of time exploring with the head-up the lower the HR ($b = -161.5$, $P \leq 0.004$).

There was no association between the proportion of time spent in any of the recorded behaviours and plasma cortisol levels.

Behaviour - Qualitative assessment

In the qualitative behavioural assessments, we recorded a significant agreement between assessors only for the conclusive motivation, signalling behaviour, signs of stress and possessive reward playing. In Table 4 are reported these paired agreements, identifying the assessor pair and the particular behaviour, and showing the value for Kappa with its significance (conclusive motivation, signalling, sign of stress, possessive reward playing). The analysis for variables indicative of decreased search time found only one variable, signal average, to be significantly associated (P = 0.009). The variable 'signal average' was coded from 1 to 5, with 5 indicating greater signalling. The analysis showed that for every integer increase in the scale the search time was reduced by a mean of 150.6 (se = 57.70) seconds.

4. Discussion

The aim of this study was to characterise the physiological and behavioural responses of military avalanche dogs to a search and rescue work. A deeper knowledge of their responses is important because these animals play a pivotal role in rescue missions, making the difference between the survival or death of a victim. The SASR induced significant increases on RBT and HR, cortisol, CK, AST, LDH and NEFA. However, only AST values were higher than the upper limit reported for healthy dogs at rest [39]. Overall, physiological changes progressively decreased to have completely recovered within two hours following a successful rescue (T3), with the exception of sera cortisol levels that showed a slower decline and also AST that was still increasing. For the baseline values (T0), most dogs had blood values, RBT and HR within published reference ranges (70-120 bpm, 37.9 - 39.9°C) [39]. Some individuals showed a peak of HR soon after the descent from the helicopter (T1) indicating a fast activation of the sympathetic-adrenal-medullary axis as a component of the stress response. The range of HR and RBT increases were similar to those observed in healthy Labrador Retrievers 5 minutes post-exercise during training and field trial competition [40]. Heart rate rises could reflect that the SASR stimulated an "emotional tachycardia" as shown in other species [25], often also associated with body temperatures increases. These changes might be an adaptive response to the SASR, but also a consequence of the dogs' increased locomotor activity. Similar changes were observed in military dogs during a three-day terrestrial search operation in response to physical and mental challenge [41] and after 20 minutes of physical activity on a treadmill in explosive detection dogs [7].

In agreement with Bergeron et al., [42], the elevated heart rate values at baseline sampling (T0, 116.20 bpm) might indicate the presence of a high level of arousal in the SAGF dogs possibly elicited by the novelty of blood sampling procedure. SAGF dogs did show overt signs of excitement when the operator and the handlers entered the area outside their living pen. This elevated HR baseline values might also reflect the excitement of the anticipation of the search, an emotional response to learning known as “Eureka effect” [43], where dogs showed an anticipatory response when experiencing a learning process after recognition of the assigned operational task. It is probable that the SAGF dogs were experiencing a similar excitement. They were daily trained, and showed always signs of excitement when their handlers in the morning let them out of their living pen to work. They were accustomed to this practice, thereby this could have represented a cue of anticipation of the search work.

Cortisol baseline values detected in our study were similar to those reported in other studies on working dogs [20]. The SASR induced an increase of the adrenocortical response in the SAGF dogs soon after helicopter transport (T1) reaching peak values after the SASR (T2). However, this increase of plasma cortisol levels was very moderate, values remaining always within the normal range compared to other studies in dogs [8][44]. Cortisol increase showed a tendency to progressively decline over the time, even though not until a complete recovery after 2 hours from the end of the SASR (T3). Haverbeke et al., [8] registered a temporary increment of cortisol levels in a group of military dogs submitted to consecutive training sessions, but this was temporary and declined back to baseline after challenge as a sign that dogs might have a reduced welfare, but were not chronically stressed.

Similar trends in the cortisol response, comparable to those of our study, had been observed following intensive work in search and rescue dogs [41], in search and rescue dogs subjected to physical exertion and working in adverse weather conditions (cold and heat stress) and after exercise [13], in dogs after playing [45] and short-term challenges [27], as well as in explosive detection dogs as a response to an emotional reactivity test [46].

Plasma CK, AST and LDH activities are measured to assess the degree of metabolism and muscular effort. We recorded a significant increase of CK activity during the experimental period compared with baseline values. Knob and Seidl, [47] observed that the normal range of CK enzyme activity was higher in dogs than humans, and considered a normal reference CK range up to 84 U/L. That the peak of CK activity occurred immediately after the helicopter trip may indicate that loading and transport induced an intense muscular stress. Since CK is the first enzyme to appear in the blood as a result of damage of muscle cells and just as quickly it tends to disappear [48], this temporary rise in CK activity could indicate the presence of an acute state of muscle exertion in the SAGF dogs. Also Schneider et al., [41] observed that the CK activity was the most obvious change among the measured haematological variables after physical stress. SASR also induced a progressive increase of AST activity over the time, but this was very moderate, confirming that the physical effort in the SAGF dogs was not excessive enough to cause substantial muscle damage. Previous studies showed that only elevated increases of AST activity after exercise were associated with damage to the muscle cell membranes [49][50]. Conversely, similar to CK activities, LDH rose after helicopter transport also implying an intense muscular effort. However, LDH activity tended to return to baseline values after the period of rest (T3), suggesting a fast recovery in the muscle metabolism. Similarly, Rovira et al., [14] found that exercise induced elevation in plasma CK, AST, LDH activity and cortisol concentrations, that returned to resting values after 30 minutes of a passive recuperation in search and rescue-trained dogs. Plasma NEFA levels rose moderately above baseline values soon after the helicopter trip (T1), and the SASR (T2), and then progressively declined at rest (T3), suggesting fat mobilization to produce energy. High plasma NEFA concentrations after exercise play an important role in the regulation of the oxidative metabolism by promoting muscle glycogen resynthesis by increasing glucose availability and inhibiting muscle glycolysis through the glucose-fatty acid cycle [51]. Large elevation of plasma NEFA levels post-exercise has been previously reported in dogs after a 4 hour field search [15].

Overall, we recorded only moderate and temporary changes in the physiological parameters suggesting that search and rescue activities involved in the SASR elicited only a short-lasting stress response in the avalanche SAGF dogs. This will have been evoked by the additive factors within the SASR (helicopter transport, winching to the ground, physical effort during the search and rescue work). The overall response remained within the physiological limits and was also limited over the time. This was quite remarkable when considering the extreme, harsh environmental and working conditions (environmental air temperatures ranged from -8.5 to -10.4°C with 28% humidity and a wind-chill effect of -29°C) to which the SAGF dogs were exposed and indicate good adaptation to these conditions by the SAGF dogs. In this study they demonstrated excellent search performances, probably due to a good physical training program that allowed them to tackle the search task without showing particular signs of stress or fatigue. In a previous study we found that SAGF dogs were able to recover within 2 hours following the exercise stress represented by the SASR, as shown by the return to pre-SASR values of the expression of metabolism and oxidative stress-related genes [33]. This appears to confirm that the physical training these military dogs undergo prepared them well for ASR, enhancing their performance and capacity to potentially repeated high-intensity effort. This finding is highly desirable from the standpoint of the operational use of these animals, as if dogs do not show early signs of fatigue after searching for the first missing person, they could be used in succession for the discovery of other buried victims, to successfully complete other searches during the same avalanche rescue mission. Haverbeke et at., [8] found that military working dogs had a good adaptation capacity to an additional challenge, thereby might have a less diminished welfare than supposed. Rooney et al., [52] found that working dogs that showed high levels of physiological stress were also those that tended to perform poorly during training.

The analysis of the videos made during the avalanche simulated search missions gave further insights. As regards the behavioural, quantitative assessment of the performance of the SAGF dogs during the SASR, it was found that duration or frequency of analysed behaviours did not differ in relation to time of search. Therefore, it did not highlight any predictive behaviour associated with a shorter, successful search. This could be due to the lack of variability of the search time amongst all the dogs: a maximum search time of only 564 seconds. As would be expected, exploring (both with the head down and up) and running were recorded as the most prevalent behaviours during the search.

In some SAGF dogs high baseline values for RBT and HR were recorded that could perhaps reflect an anticipatory response to the excitement of the search mission, indeed HR at T3, recovery, was actually notably below heart rate at T0, baseline. It is common practice for a handler to stimulate an increase in arousal to prepare a dog for the work ahead. The average fluctuations of RBT in the SAGF dogs were always within published normal reference range, as suggested by Steiss et al., [40]. However, we observed a significant relationship between RBT and the proportion of time spent running and walking. This is likely to be a result of the internal physiological adjustments, keeping a constant core temperature to balance the extreme climatic conditions, which would tend to cool external body temperature. The impact of these climatic conditions on the RBT of the dogs is not clearly established. Ambient temperature seemed to not have a marked effect on average rectal temperatures, but might influence their thermoregulatory ability when dogs perform less strenuous behaviours, such as walking. Steiss et al., [40] reported a wider range of fluctuations of values of rectal temperature in Labrador Retrievers dogs during field trial training and competition, but at higher ambient temperatures (29-30°C).

In the SAGF dogs, in part the HR response could be also constituted by a secondary effect of increased physical activity involved in the SASR. Similar heart rate responses have been reported in dogs that were subjected to a variety of conditions, such as different types of stimuli [17][23], human-animal interaction effects [53]. Bergamasco et al., [53] indicated that both sympathetic arousal and vagal tone alterations resulting from confrontational, environmental challenges could influence cardiovascular function and subsequent HR in dogs. This might explain the lower HR values recorded in relation to a longer time spent standing still with the head up and exploring with the head up in the SAGF dogs.

The behavioural qualitative assessment of the search performance of the SAGF dogs showed that the agreement among the scores of dog performance during search by the handler and the two observers was poor. However, most of the time overall scores tended to slip by only one category, indicating that differences were not large. So it seemed that individual opinions still largely influenced the behavioural assessment of the dogs and that better training of the observers would be required to improve agreement. In a previous study, good agreement was found between subjective ratings of the search performance of the dogs made by dogs' trainers and that of a scientist expert on dog behaviour [31]. These authors pointed out the importance of dogs' handlers being able to empirically judge the ultimate search performance of their dogs, including potentially critical aspects, which might cause a delay in the discovery of the missing victim.

Not one single particular behaviour seemed to be related to the search speed of the dogs. However, all these dogs had been selected and similarly trained to find people. The only exception was signalling: the more intensively dogs did this, the faster they had found the buried victim. Further investigation of these results might be fruitful, to investigate if good signalling could be predictive of better search performance, or whether it was simply correlated with the chance early discovery of the victim.

This study encompassed a small group of dogs, which had already been selected by the same military school and trained for the same work tasks using a similar method. So, they were necessarily relatively homogeneous. A high score in signalling might indicate an easy search, however, it could be that a 'better' dog would signal more. In this study each dog was only observed during one search, repeated searches by the same dogs would be required to further investigate this effect. A larger sample of dogs should be investigated for highlighting possible breed or sex differences.

In our study we could not explore the time-physical effort relationship. However, notwithstanding the arduous environmental and working conditions, all avalanche SAGF dogs completed their search within about 10 minutes, which was remarkably fast considering the size of the simulated avalanche area. This result was particularly relevant considering that there is an earlier and quicker drop in survival at the early stages of burial (10-18 minutes) of avalanche victims [54][55][11].

Behavioural and physiological responses could reflect different coping styles to deal with stressful situations [28][56]. From our findings, this group of SAGF dogs seemed to adopt mainly proactive coping styles characterized by active attempts to counteract the stressful stimuli, low HPA-axis reactivity [28] and high activation of the sympathetic-adrenomedullary system [57]. This might be more functional, enabling them to reach higher search performance success, resulting not only from the dogs' personality factors but also from the training process and from other rearing experiences [58][27].

Notwithstanding some individual variability, overall high levels of fitness and performance were observed in the dogs. This could reflect good dog selection and training conditions, and a positive handler-dog relationship, predisposing dogs' wellbeing in working conditions.

The SAGF dogs' quick recovery indicates the possibility for their safe use in more extended, multiple search and rescue missions. Improving the performance of avalanche search and rescue dogs implies improving rescue times and increasing the number of successful rescues. However, the recovery of the dogs following more extended, multiple search and rescue missions should form the basis of further study to explore the limits of their performance and endurance. The group of working dogs used in this study were in fine tune with their handlers. Their success as a team of two was in large part due to extended training, itself a highly time-consuming and demanding task. Great attention should be paid to the wellbeing of these dogs given the value of their work and the potential of their work environment to degrade their welfare. Overall the findings of this study suggest that the dogs and the training that they have received have resulted in an animal well adapted to the task that they are required to perform.

4. Conclusion

The simulated avalanche search and rescue mission induced temporary and adaptive physiological and behavioural responses in the SAGF dogs. Overall, this group of military dogs carried out high performance search tasks under extreme conditions. However, broader studies would be needed for better investigating the impact of search and rescue missions on avalanche military dogs globally. All SAGF dogs successfully found the buried victim within 10 minutes, which coupled with their quick physiological recovery, suggest their safe use in multiple avalanche search and rescue missions. Findings of this study indicated SASR did not compromise military dogs' welfare, notwithstanding the highly demanding task imposed in extremely difficult environmental conditions.

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Fig. 1 Experimental design

Figure 2: Duration (in seconds) of the simulated avalanche search and rescue mission (SASR) (including the last 10 seconds of digging) in individual SAGF dogs.

Figure 3: Repeated physiological data recorded on SAGF dogs at different sampling times (T0, T1, T2 and T3.
Graphs show the physiological observation (Temperature, Heart Rate, Cortisol, Creatine Kinase, Lactate Dehydrogenase, NEFA, Aspartate Aminotransferase) on each SAGF dogs (n=15) at different sampling time (T0, T1, T2, T3). Gray lines represents the repeated measures for the physiological data with the best fit regression line superimposed (in black).

Figure 4. Duration and frequency of behaviours in SAGF dogs during the simulated avalanche search and rescue mission (SASR).
a) time spent (duration) for each behavioral variable from SAGF dogs during search mission.
b) total number of occurrences (frequency) for each behavioral variable from SAGF dogs during search mission.

Table 1: Demographic of the SAGF dogs

Table 2: Behavioural variables measured from dogs during the simulated avalanche search and rescue mission (SASR)

Table 3: Simulated avalanche search and rescue mission (SASR) ability: SAGF dog's characteristics rated by the handler and two observers after the *SASR*.

Table 4: Simulated avalanche search and rescue mission (SASR) ability - Qualitative Behaviour Assessment: Agreement Kappa Test between the handler (H) and the two observers (O1 and O2)

Figure 1

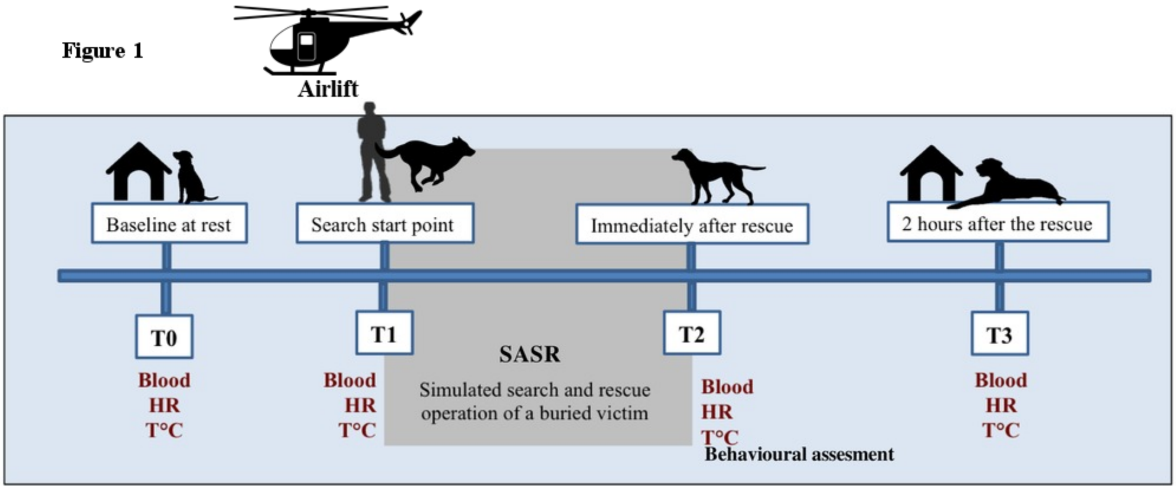

Figure 2

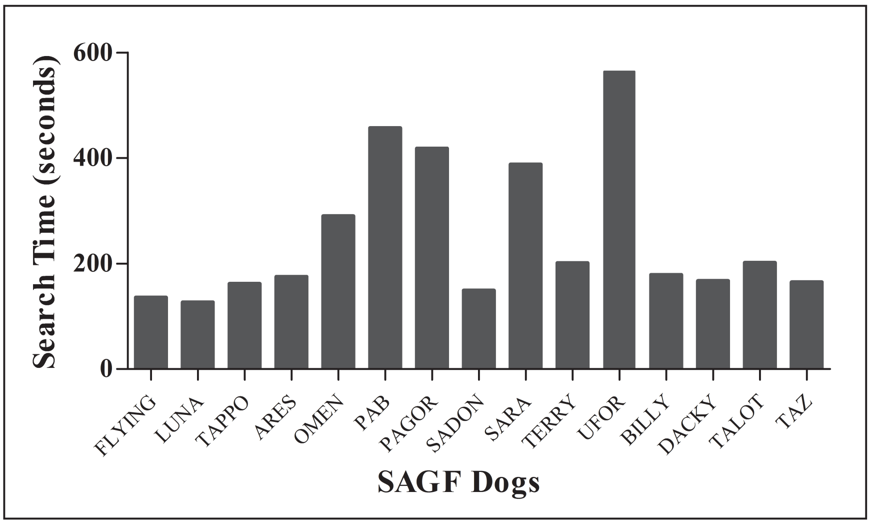

Figure 3

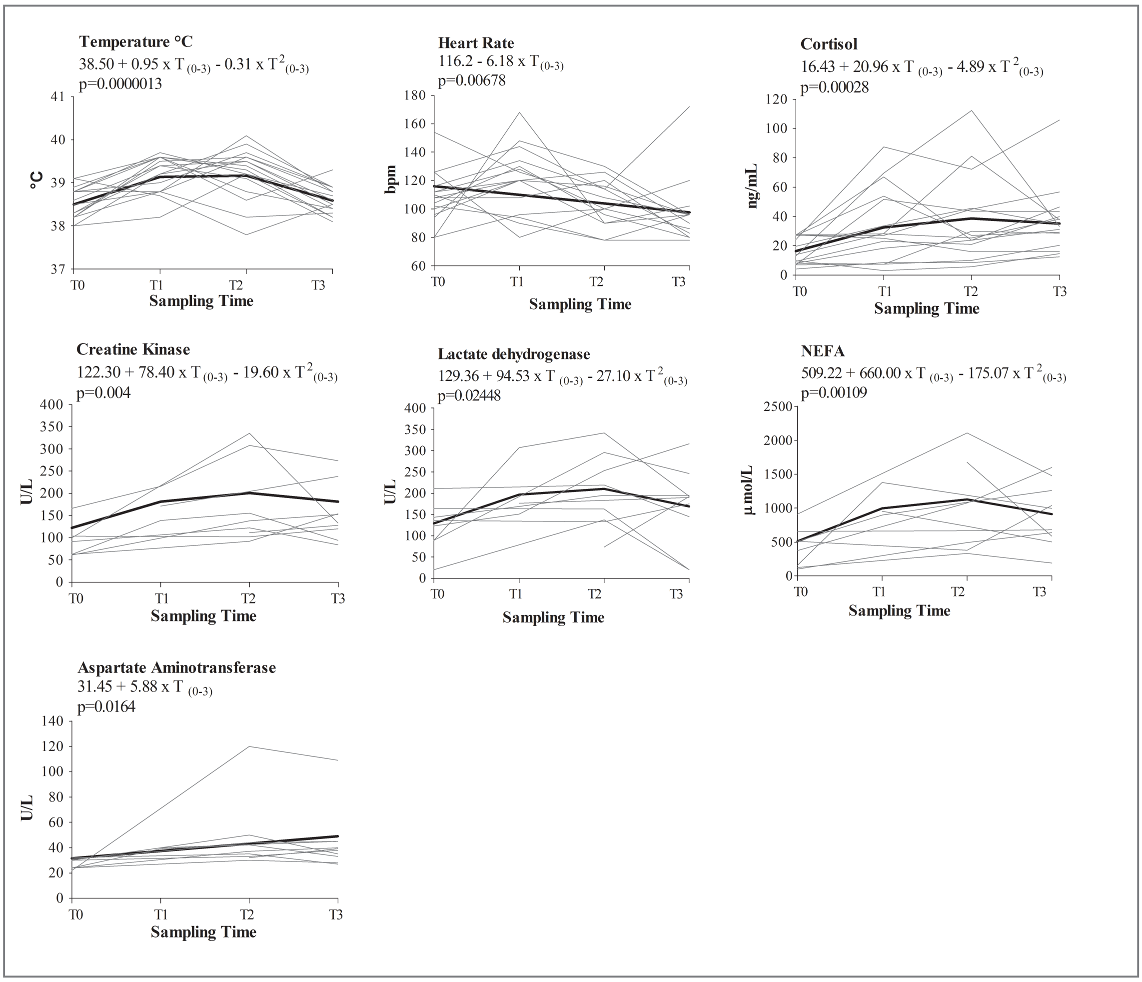
Temperature °C
38.50 + 0.95 x T (0-3) - 0.31 x T2 (0-3)
p=0.0000013
°C
Sampling Time
Heart Rate
116.2 - 6.18 x T (0-3)
p=0.00678
bpm
Sampling Time
Cortisol
16.43 + 20.96 x T (0-3) - 4.89 x T2 (0-3)
p=0.00028
ng/mL
Sampling Time
Creatine Kinase
122.30 + 78.40 x T (0-3) - 19.60 x T2 (0-3)
p=0.004
U/L
Sampling Time
Lactate dehydrogenase
129.36 + 94.53 x T (0-3) - 27.10 x T2 (0-3)
p=0.02448
U/L
Sampling Time
NEFA
509.22 + 660.00 x T (0-3) - 175.07 x T2 (0-3)
p=0.00109
µ mol/L
Sampling Time
Aspartate Aminotransferase
31.45 + 5.88 x T (0-3)
p=0.0164
U/L
Sampling Time
T0
T1
T2
T3

Figure 4

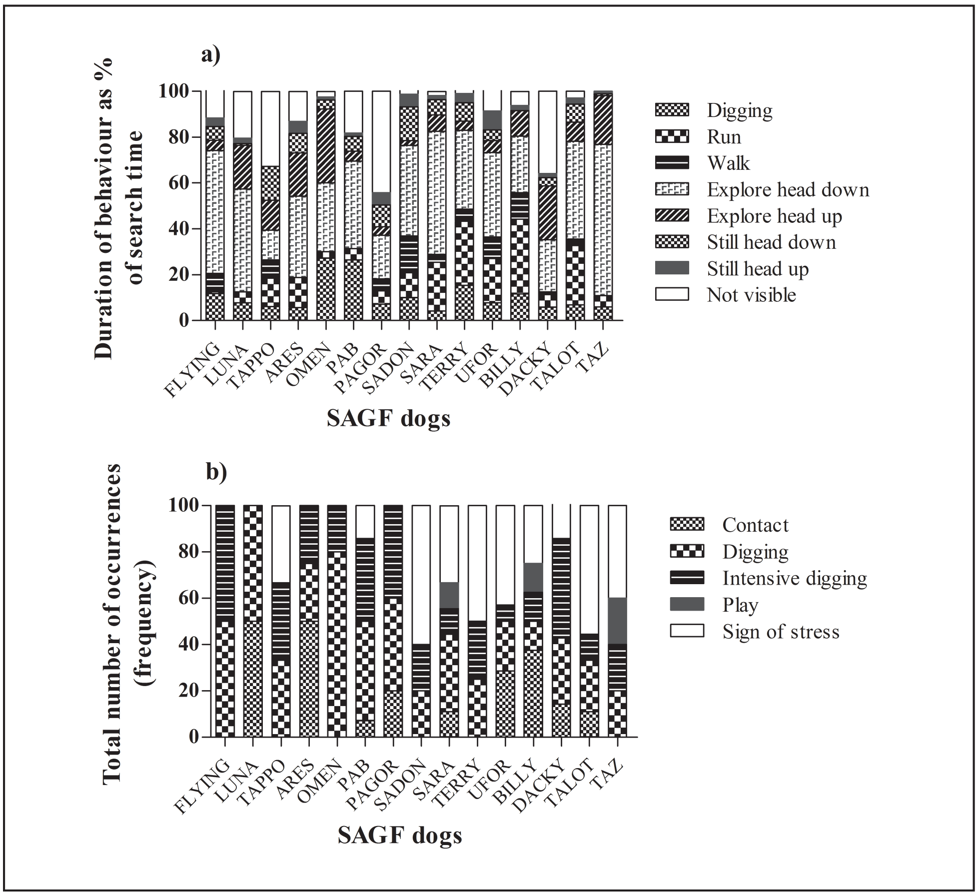
a)
Duration of behaviour as % of search time
100
80
60
40
20
0
FLYING
LUNA
TAPPO
ARES
OMEN
PAB
PAGOR
SADON
SARA
TERRY
UFOR
BILLY
DACKY
TALOT
TAZ
SAGF dogs
Digging
Run
Walk
Explore head down
Explore head up
Still head down
Still head up
Not visible
b)
Total number of occurrences (frequency)
100
80
60
40
20
0
FLYING
LUNA
TAPPO
ARES
OMEN
PAB
PAGOR
SADON
SARA
TERRY
UFOR
BILLY
DACKY
TALOT
TAZ
SAGF dogs
Contact
Digging
Intensive digging
Play
Sign of stress

Table 1: Demographic data of the SAGF dogs.

Age (years)	N°	Sex	N°	Breed	N°
1-3	6	Male	15	*German Shepard*	8
3.1-6	5	Female	2	*Belgian Shepard Malinois*	6
6.1-8	4			*Border Collie*	3

Table 2: Behavioural variables measured from dogs during Simulated Avalanche Search and Rescue mission.

Variable	Definition
Duration DIGGING	Time spent scratching with front paws on snow within the search area (in seconds)
Duration RUN	Time spent running within the search area (in seconds)
Duration WALK	Time spent moving position within the search area (in seconds)
Duration EXPLORE HEAD DOWN	Time spent exploring with head down within the search area (in seconds)
Duration EXPLORE HEAD UP	Time spent exploring with head up within the search area (in seconds)
Duration STILL HEAD DOWN	Time spent stationary with head down within the search area (in seconds)
Duration STILL HEAD UP	Time spent stationary with head up within the search area (in seconds)
Frequency of CONTACT	Number of times dog get in contact with his handler within the search area
Frequency of DIGGING	Number of times dog scratch with front paws on snow within the search area
Frequency of INTENSIVE DIGGING	Number of times dog intensively scratch with front paws on snow within the search area
Frequency of PLAY	Number of times dog plays within the search area
Frequency of SIGN OF STRESS	Number of times dog shows signs of stress* within the search area

* miscellaneous of stress-related behaviours (panting, barking, paw-lifting, snout and lip-licking, circling, body shaking, yawning)

Table 3: Simulated avalanche search and rescue mission (SASR) ability: SAGF dog's characteristics rated by the handler and two observers after the SASR.

No.	Characteristic of the SAGF Dog	Description
Rating scale 1-5		
1	Attention	Attentional state during search
2	Initial Motivation	Motivational state at the onset of the search
3	Conclusive Motivation	Motivational state at the end of the search
4	Signalling	Digging and barking aimed to signal the *disperse victim*
5	Sign of stress	Dog showing a range of stress-related behaviours*
6	Possessive reward playing	Dog reluctant to return the reward to the handler
7	Collaborative reward playing	Dog playing to leave and take again the reward with the handler
8	Relationship with the handler – Communication	Dog-handler communication
9	Relationship with the handler – Collaboration	Dog-handler collaboration
Answer Yes/NO		
10	Urination	
11	Defecation	
12	Urine marking	
13	Play with the 'disperse victim'	
14	Play with the handler	

* stress-related behaviours (panting, barking, paw-lifting, snout and lip-licking, circling, body shaking, yawning)

Table 4: Simulated avalanche search and rescue mission (SASR) ability - Qualitative Behaviour Assessment: Agreement Kappa Test between the handler (H) and the two observers (O1 and O2).

Characteristic of the SAGF Dog	Inter-rater H-O1-O2	Kappa coeff	Kappa P<
Initial Motivation	ns	ns	ns
Conclusive Motivation	O1-O2	0.3924	0.0025
Signalling	H-O2	0.2537	0.0319
	O1-O2	0.5479	0.0002
Sign of stress	O1-O2	0.5894	0.0000
Possessive prey playing	H-O2	0.2641	0.0098
Collaborative prey playing	ns	ns	ns
Relationship with the handler – Communication	ns	ns	ns
Relationship with the handler – Collaboration	ns	ns	ns
Urination	ns	ns	ns
Defecation	ns	ns	ns
Urine marking	ns	ns	ns
Play with the disperse victim	ns	ns	ns
Play with the handler	ns	ns	ns